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Sodium hypochlorite with reduced surface tension does not improve in situ pulp tissue dissolution

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Abstract: INTRODUCTION: Sodium hypochlorite (NaOCl) solutions with added wetting agents are advertised to dissolve necrotic tissue in root canals faster than their counterparts without a lowered surface tension. This was tested in the current study, and the null hypothesis formulated was that there was no difference between a commercially available NaOCl solution with a lowered surface tension (Chlor-XTRA; Vista Dental Products, Racine, WI) and a counterpart containing the same amount of available chlorine without added wetting agents regarding the soft tissue that remains in oval-shaped canals after mechanical preparation and irrigation. METHODS: Formerly vital extracted teeth (N = 44, 22 pairs) with similar anatomy were radiographically paired and chemomechanically prepared. In 1 tooth from each pair, a 5.25% NaOCl solution with reduced surface tension was used; in the other, a pure, technical-grade NaOCl solution of 5.25% was used. The percentage of remaining pulp tissue (PRPT) was histologically assessed in root cross-sections. The non-Gaussian raw data were subjected to Kruskal-Wallis and Mann-Whitney U tests to verify the respective effect of the cross-section level and solution on the PRPT. The relationship between the cross-section level and the PRPT was estimated by the Spearman correlation test. The alpha-type error was set at 5%. RESULTS: The cross-section level significantly influenced the PRPT ($P < .05$), whereas the PRPT was not influenced by the solution used ($P > .05$). A significant inverse correlation was found between the cross-section level and the PRPT ($P < .05$, $r = -0.330$). The lower the distance to the apex, the higher the PRPT regardless of the solution used. CONCLUSIONS: Contrary to the advertised statement, the dental solution with a reduced surface tension did not dissolve vital pulp tissue in oval root canals any better than a conventional NaOCl solution of similar strength. Closer to the apex, pulp tissue dissolution is less efficient irrespective of the solution.

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NaOCl with Reduced Surface Tension Does not Improve in situ Pulp Tissue Dissolution

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NaOCl with Reduced Surface Tension Does not Improve *in situ* Pulp Tissue Dissolution

Introduction

The so-called chemo-mechanical principle is the basis of root canal debridement (1). This concept, originally deduced from wound debridement in WW1 (1; 2), was confirmed by the clinical observation that neither mechanical nor chemical cleansing per se suffice to render root canals of teeth with apical periodontitis free of bacteria and soft tissue remnants (3; 4). The more complex the anatomy of root canals, the harder it is to clean them (5). The chemical aspect of root canal treatment relies on sodium hypochlorite irrigation (1). As has been shown, NaOCl (or more precisely, OCl^-) has unique soft tissue-dissolving properties (6; 7) and thus has prevailed as the main endodontic irrigant for almost a century (8).

After the inception of NaOCl to endodontic treatment, many approaches have been made to enhance its effectiveness. Soft tissue lysis by NaOCl is influenced by irrigant concentration, temperature, contact time, agitation, contact area, and pH (9). In addition, it has been claimed that surface tension may also play a role in this context. It has been shown that narrow canals are better permeated with an irrigant of lowered surface tension compared to a normal aqueous counterpart (10). However, these original experiments were performed with extracted teeth of unknown water content. In the clinical situation, the root canal space is inherently wet (11). It is thus questionable whether surface tension (and thus wetting ability) of endodontic irrigants play any role (12). Controlled in vitro experiments have shown that soft tissue pieces immersed in NaOCl solutions are dissolved irrespective of the surface tension of the irrigant (13). However, soft tissue dissolution properties of unaltered versus surface tension-reduced NaOCl solutions have never been compared in human root canals. This was done in the current study using extracted, formerly vital, teeth with oval root canals.

The Null hypothesis tested in this study was that there was no difference between a commercially available NaOCl solution with lowered surface tension (Chlor-XTRA, Vista Dental Products, Racine, WI, USA) and a counterpart containing the same amount of available chlorine without added wetting agents regarding the soft tissue that remains in oval-shaped canals after mechanical preparation and irrigation.

Materials and Methods

NaOCl Solutions

Three bottles of Chlor-XTRA (Vista Vista Dental Products, Racine, WI) of different batches were bought from a dental supplier in the USA. For comparison, technical-grade 5.25% NaOCl solution was prepared by diluting a 10% NaOCl solution (Merck S.A., São Paulo, SP, Brazil) with ultrapure water in order to produce a pure technical control solution. Its pH was adjusted to 11 with 1 N HCl. The final concentration of the technical NaOCl solution was verified iodometrically (9).

Surface Tension Assessment

Teflon flat standardized surfaces were used to test both experimental solutions and also the control with water (5 drops per solution in each surface). The contact angles of both solutions were measured using a goniometer device Phoenix 300 Plus (SEO – Surface Electro Optics, Swon City, Gyunnggido, Korea), with an 6.4 internal zoom coupled to a ~0.45 µm/pixels resolution digital camera controlled by the Image XP software (SEO). The experiment was performed under standard conditions of temperature and relative humidity. A 5 ml Luer-lock disposable syringe, filled with distilled water, with a 23G needle with a cut tip, was inserted into the specific holder of the goniometer. The Teflon surface sample was placed at the specimen stage, under the syringe, with the central area aligned with the needle. A computer controlled automatic syringe system was used to dispense a 2-3µL droplet over the surface. A digital image of the droplet's silhouette was captured and measurements of volume and contact angles (right and left) were automatically performed by Image XP, with an accuracy of $\pm 1^0$. This procedure was conducted on five different areas of each Teflon surface. After determination of contact angles, right and left angles of each drop were averaged. Drops with differences over than 10 units were considered as experimental error and were removed from the analysis and the procedure was redone.

In-vivo prospective selection process of vital teeth

One hundred fifty two adult subjects voluntarily participated in the present study, which was reviewed and approved by the Ethics Committee. All teeth were scheduled for extraction due to advanced periodontal disease or non-restorability. Only vital single-rooted teeth were included in the present study. To ensure pulp vitality, teeth were first tested using Green Endo-Ice refrigerant spray (Hygenic, Akron, OH). Teeth that responded positively were then anesthetized and accessed to verify the presence of a bleeding pulp. Teeth that failed to provide the above dual proof for vital pulp were excluded from the study. It took 10 months to collect 136 single-rooted teeth with confirmed vital pulps. Following extraction, each tooth was immediately placed into a vial labeled with a 4-digit alphanumeric code and containing 10 ml of buffered 10% formalin. Radiographs were taken in bucco-lingual and mesio-distal projections to select only teeth with a single root canal and to categorize them as either oval or circular-shaped. Root canal diameters were measured at level 5 mm from the apex through image analysis software (AxioVision software 4.11, Carl Zeiss, Germany); when the bucco-lingual diameter was at least 2.5 times larger than the mesio-distal diameter, the canals were classified as oval-shaped. All round-shaped canals, in which the mesio-distal diameter was similar to the bucco-lingual diameter, were excluded from the present study. All teeth presenting an isthmus, lateral, accessory or two canals were also excluded from the study. Furthermore, only root canals with an initial apical size equivalent to a size 10 K-file were included.

This selection process resulted in 54 vital mandibular canines that met all of the above criteria. From this collection, 22 pairs of teeth (n = 44) were radiographically pair-matched. Subsequently, one tooth from each pair was randomly assigned to one of the two groups in this study. After the groups were established, a flip of a coin was used to define which teeth would be treated according to which protocol (G1: Chlor-XTRA, G2: 5.25% NaOCl). Ten teeth were used as positive controls (no treatment, maximum amount of pulp tissue).

Root canal preparation and irrigation

A custom-made epoxy resin fitting was employed to close the apex of each tooth. Tooth length was standardized to 18 mm by cutting part of the crowns, and the root canal patency was confirmed by inserting an ISO size-10 instrument. Working length was established so that roots were prepared to the apical foramen. The same operator performed all preparation procedures. Teeth were prepared with the ProTaper Universal instruments driven at 300 rpm with 2 N·cm of torque (XSmart, Dentsply-Maillefer, Ballaigues, Switzerland). The sequence followed was: SX file (1/2 of the WL); S1 file (1/3 of the WL); S2 file (2/3 of the WL); and F1, F2 and F3 instruments (full WL). Shaping X, S1 and S2 were used with a brushing motion according to the anatomy of each root canal.

Irrigants were continuously delivered by a VATEA peristaltic pump (ReDent-Nova, St. Ra'anana, Israel) at a rate of 3 mL/min connected to a 30-gauge Endo-Eze Tip (Ultradent Products Inc., South Jordan, UT, USA) placed passively into the canal, up to 2 mm from the apical foramen without binding. The aspiration was performed with a SurgiTip (Ultradent) attached to a high-speed suction pump. Between each file, root canals were irrigated with 3 mL of irrigant for 2 minutes. As a result, a total volume of 18 mL of NaOCl per root canal was used during the mechanical preparation. An additional rinse was performed after the shaping of the root canal had been completed; 12 mL of solution during 20 min. This way, a standardized total volume of 30 mL of irrigant was used during 30 min. The smear layer was removed with 3 mL of 17% ethylenediamine tetraacetic acid (EDTA; pH 7.7) for 3 minutes (3 mL/min). Three mL of bi-distilled water was used for 3 minutes as a final rinse. All canals were dried with paper points (Dentsply-Maillefer). One experienced operator - blinded regarding the irrigant used - performed all preparations.

Histological assessment

Specimens were immediately immersed in 10% buffered formalin for 48 hours and then demineralized in a 22.5% (vol/vol) formic acid solution and a 10% (wt/vol) sodium citrate solution for a period of 2 to 3 weeks. The end point was monitored radiographically. After rinsing for 24 hours in tap water, the specimens were dehydrated and processed for routine histological examination. Teeth were embedded in paraffin blocks, and serial 0.6 µm thick cross-sections were obtained every millimeter from the 1-5 mm apical levels. This resulted in a total of 5 slides per tooth. Sections were mounted on glass slabs and stained with hematoxylin-eosin.

Morphometric evaluation

The specimens were visualized using an Axioplan 2 Imaging fully-motorized light microscope (Carl Zeiss Vision, Hallbergmoos, Germany). Image analysis and processing were completed using the Axion Vision image 4.5 Zeiss system (Zeiss) to trace the outline of the area of interest. In this way, the cross-sectional area of each root canal and the remaining pulp tissue were measured (μm^2). Further, the percentage of remaining pulp tissue area (PRPT) was calculated for each root canal section by dividing the remaining pulp tissue area by the total area of the root canal in the same section. The operator who made the measurements was blinded as to which samples were matched to which solution and all the measurements were repeated twice to ensure reproducibility.

Statistical analysis

Firstly, contact angle data were submitted to a normality test (Kolmogorov-Smirnov), which revealed a bell-shaped distribution ($P>0.05$). Therefore, a one-way ANOVA procedure was carried-out to verify differences between NaOCl, Chlor-XTRA and water. As the Levene test had demonstrated non-homogeneity of the variance ($P<0.05$), the Tamhane post-hoc test was used for pair-wise comparisons. Drops over the Teflon surface were averaged and the single value obtained per solution used for descriptive analysis. Data were expressed as degree of contact angles.

In order to validate the pair-wise radiographic matching of samples, the total area of the root canal at the cross-sections was compared between the groups, using a Student's *t*-test. Further, statistics were used to verify the effect of the level of cross-section and solutions on the PRPT. Because preliminary analysis of the raw pooled data showed a non-Gaussian distribution (Shapiro-Wilk normality test) and homogeneity of the sample (Levene test), non-parametric tests were selected. Kruskal-Wallis test was used to verify the effect of cross-section level on the

PRTP. Mann-Whitney U test has been used to determine the difference in PRTP influenced by the solutions. At last, the relationship between the cross-section level and PRTP was estimated by a Spearman correlation test. The alpha-type error was set at 0.05 for all analysis. SPSS 17.0 (Statistical Package for Social Sciences, Santo Antonio, USA) was used as the analytical tool.

Results

All microscopic images for the histological control group displayed large amount of residual pulp tissue. Thus, this control group confirmed the experimental histological model and also the prospective in-vivo collection of the specimens.

As indicated by the contact angle on Teflon surfaces, Chlor-Xtra (59.1° ± 2.57) had significantly lower surface tension than water (104.5° ± 2.34) or the technical-grade NaOCl (91° ± 8.5) ($P<0.05$). No significant difference was observed between NaOCl and water ($P>0.05$) – Figure 1.

The preliminary Student's *t*-test indicated an effective tooth matching as no significant difference was detected in root canal areas between groups ($P>0.05$). The values of central tendency regarding percentage of remaining pulp tissue area (PRTP) are presented in Figure 2. Kruskal-Wallis analysis of variance indicated that the cross-sectional level significantly influenced PRPT ($P<0.05$), whereas Mann-Whitney test did not identify any difference in PRTP between the solutions ($P>0.05$) (Figure 1). The Spearman correlation test revealed a significant inverse relationship between cross-section level and PRPT ($P<0.05$ $r_s = -0.330$). The lower the distance to the apex, the higher was the amount of PRPT in the root canal, regardless of the irrigation solution used (Fig. 1). Histological cross-sections illustrating the remaining pulp tissue in the experimental specimens are shown in Figure 3.

Discussion

Similar amounts of pulp tissue remained in the canals were found in Chlor-XTRA or 5.25% NaOCl-treated specimens. Therefore, the null hypothesis was fully accepted. In other words, Chlor-XTRA did not improve the pulp tissue chemical dissolution under the environmental conditions of the root canal space when compared to a conventional NaOCl solution with non-altered surface tension. The current study was based on the rationale that *in situ* root canals and adjacent dentin walls are liquid filled, and surface tension of liquids to be introduced thus plays a minor role in this environment. Therefore, this means that testing surface tension on dentine in dry conditions may have overestimated the effect of this physical parameter on soft tissue debridement. The current results are in line with two recently published studies, which found no impact of the surface tension of NaOCl solutions on tissue dissolution (13; 14).

A further finding of the present study was that the level of the cross-sections significantly influenced the amount of remaining pulp tissue. This is a reasonable finding and restates that the overall debridement decreases in a corono-apical direction. It is clear that remaining tissue after cleaning and shaping represents an area of the canal, which the instrument failed to reach mechanically. This also indicates that conventional NaOCl syringe-needle irrigation was unable to clean hard-to-reach areas in oval-shaped canals.

The current study is limited by the fact that it was performed on extracted teeth. The pulp tissue was fixed with formaldehyde. This lowers the solubility of soft tissues by NaOCl solutions (15). However, as all the teeth were prepared in the same manner, this could not introduce systematic error. Three further methodological aspects should be addressed here. The first one was about the prospective *in vivo* selection of vital teeth. This is an essential methodological step in assuring the credibility of the histological assessment (16, 17). Only by using a rigorous and well-controlled tooth selection can the root canal contents be standardized with a minimal standard of control (16).

The second methodological aspect was regarding special care to obtain experimental groups that were as balanced as possible in terms of anatomical features (18). Therefore, pair-matching was performed. This process, however, tended to limit the size of the experimental groups, although it may have been the only way to expose both irrigation protocols to equal levels of challenge and also boost the statistical power of the study (18). In the present study, the teeth were pair-matched based on visual examinations of bucco-lingual and mesio-distal radiographs, which also has limitations due to the bi-dimensional feature of the X-ray. In future studies, high-definition micro-tomography may be used to further improve the teeth pair-matching process using 3D images (18).

The third methodological aspect is about the histological assessment. In fact, histological methods have been used for many years to evaluate root canal instrumentation and may apparently be perceived as out-dated when compared to current micro-CT methods (18). Micro-CT provides valuable information about changes in the calcified tissues surrounding the root canal. However, it is unable to provide information about soft tissue or biofilm (18). Thus, histological evaluations may shed some light on this grey area (18; 19), providing valuable information that cannot otherwise be obtained. Conventional histology should thus be considered a complimentary tool to the micro-CT assessments of the root canal preparation.

In conclusion, it may be stated that in the current study, as many controls as possible were installed to check for any possible effect of lowering surface tension of NaOCl irrigants on soft tissue sorption from human root canals. No such effect, however, could be detected.

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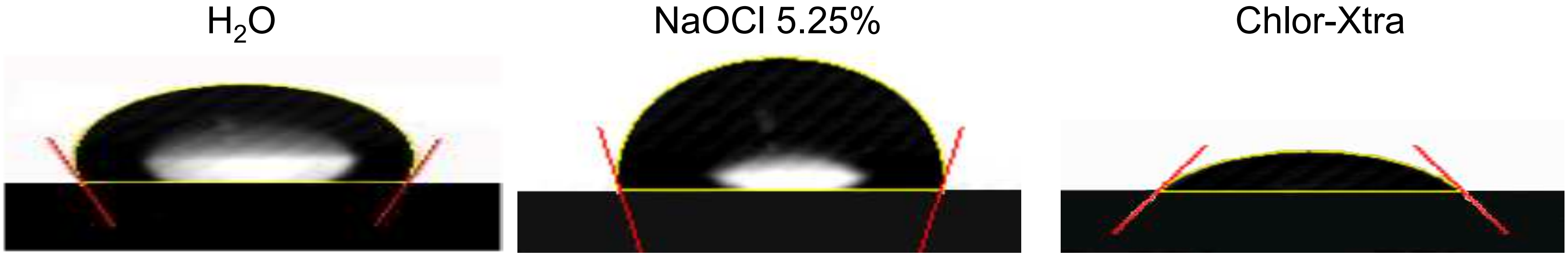
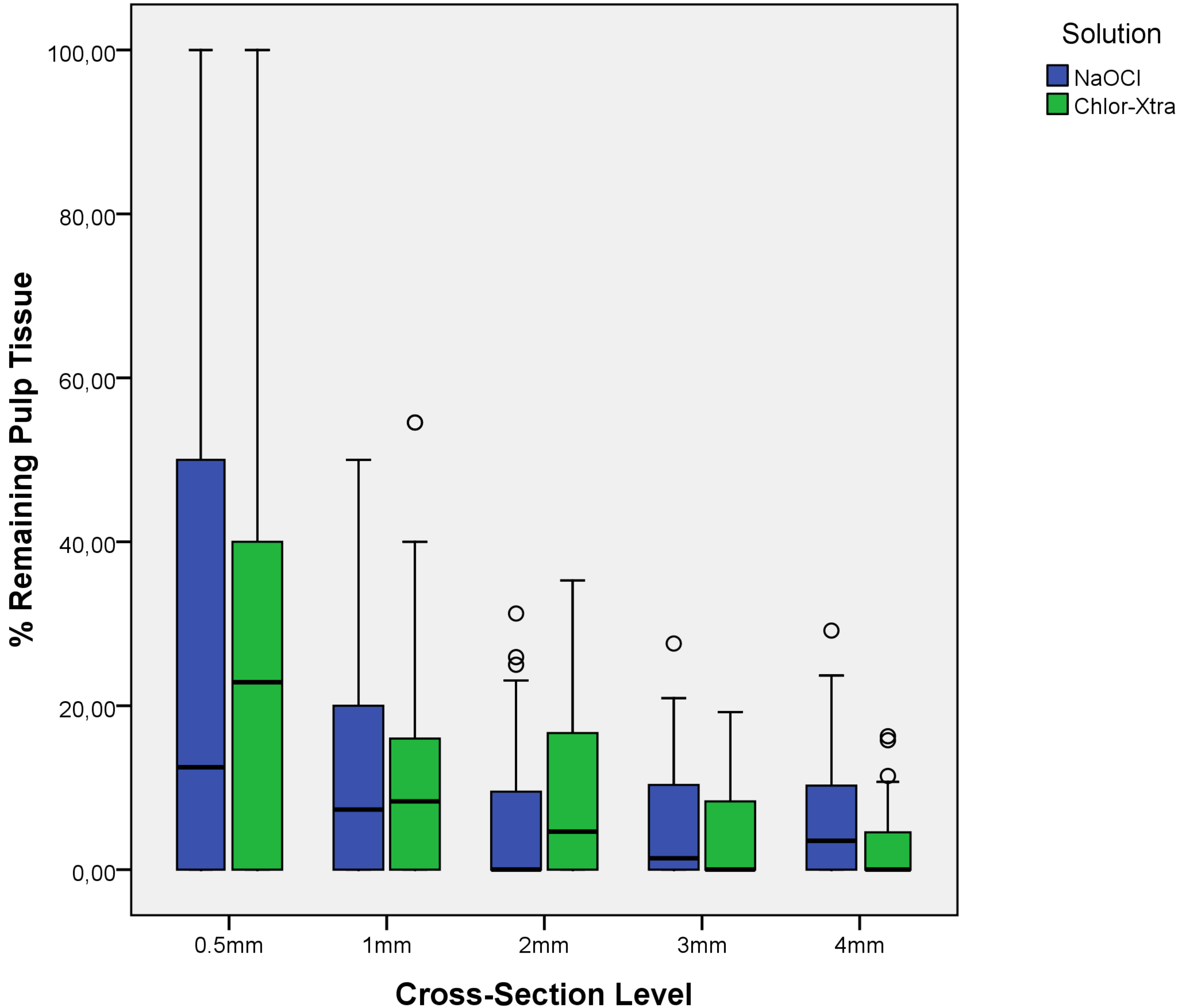
Legends

Figure 1. Boxplot of the percentage of remaining pulp tissue (PRPT) at different cross-sections levels. Observe that the lower the distance to the apex, the higher the amount of PRPT regardless of the solution tested, as indicated by the Spearman correlation test ($P<0.05$, $r_s = -0.330$).

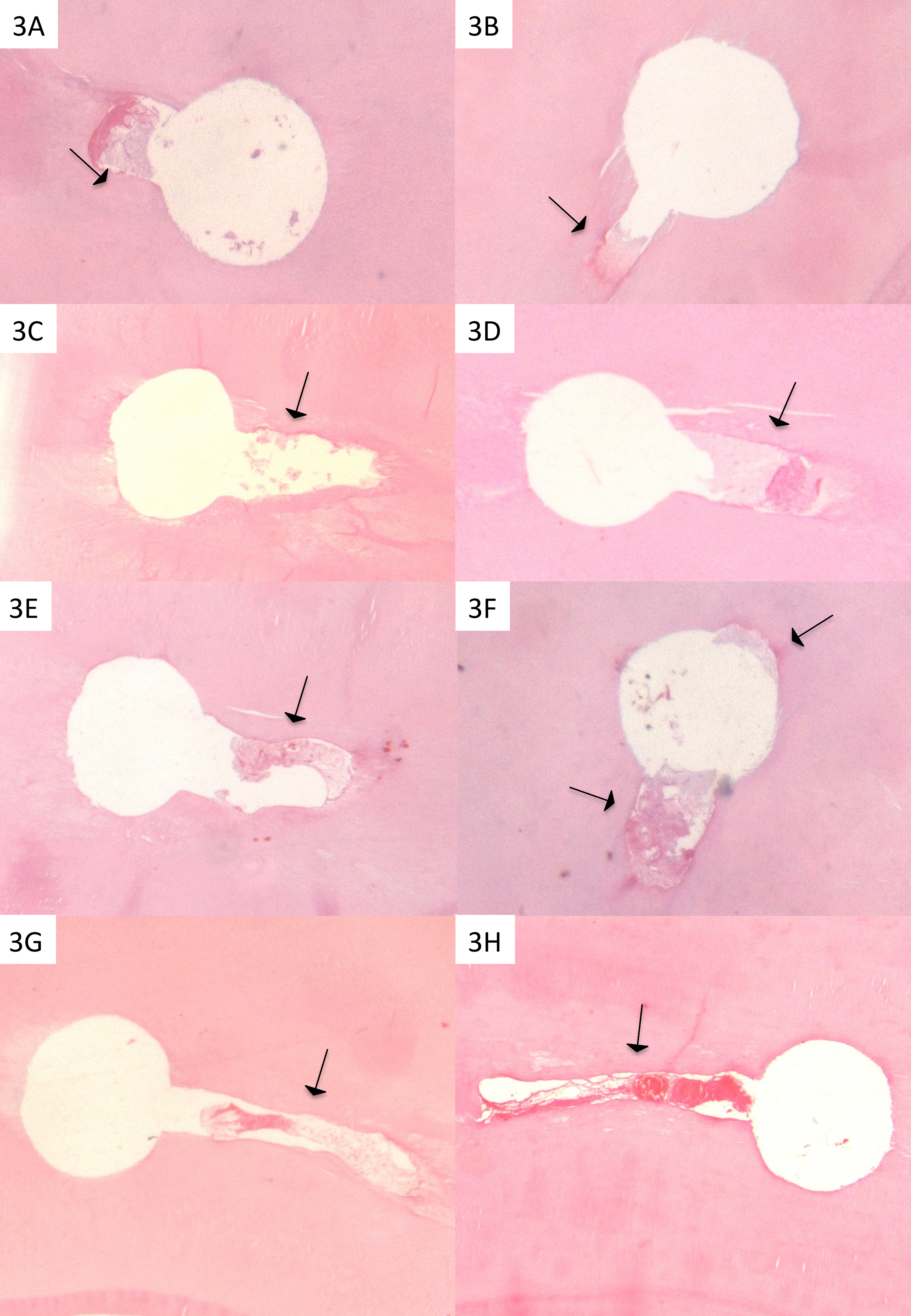
Figure 2: Contact angles of Chlor-XTRA, 5.25% NaOCl and water.

Figure 3: A, C, E and G: Chlor-XTRA-treated specimens. B, D, F and H: The counterparts - 5.25% NaOCl-treated specimens. It can be observed the presence of a significant amount of remaining pulp tissue in the unprepared buccal and lingual extensions of the oval-shaped canals. Arrows indicate the remaining pulp tissue.


H_2O
NaOCl 5.25%
Chlor-Xtra



% Remaining Pulp Tissue
100,00
80,00
60,00
40,00
20,00
0,00
0.5mm
1mm
2mm
3mm
4mm
Cross-Section Level
Solution
NaOCl
Chlor-Xtra


3A
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